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On the gross structure of an agar gel

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(WITH FOUR TEXT FIGURES)

In connection with some other work on the behavior of agar gels a peculiar appearance was noticed in certain dried strips of agar which had been swollen in water. In these cases the swollen gels, when removed from the water, would exude a considerable amount of the adsorbed liquid under slight pressure. This led to an examination under the microscope, where it was found that the gel had a decided lamellated structure. Later it was noticed that these lamellations were visible to the naked eye and could be seen when light was reflected upon the cut surface. It seemed of interest to investigate some of the causes influencing the formation of this structure, and the relation its appearance might bear to the concentration of the gel.

A brief notice of this phenomenon has already been published in D. T. MacDougal's recent contribution on hydration and growth* from data supplied by the writer.

The method of preparing the agar was that used by MacDougal and described by him in this paper, except that silk was used to cast the agar upon instead of filter paper, as the former could be more easily removed from the agar than the latter. Prepared in this way, the gels were at first allowed to stand over night in the room, and then dried at a temperature of about 70° C. It was found, however, that this first slower drying in the room had no effect on the structure, as gels put immediately into the dryer as soon as set had the same appearance. It is, of course, to be understood that the lamellations did not appear until the dried gel had been again swollen in water.

In order to ascertain whether the tension due to the drying on the silk had anything to do with the structure of the gel, a 2.5 per cent gel was cast as usual, but instead of being stretched on a frame it was cut into strips as soon as set. Some of these were hung up in the drier; others laid horizontally on the trays in the same at temperatures of about 43° C. and 70° C. Both of these showed the characteristic structure on swelling. Similar gels were dried at room temperature, 21–25° C., both stretched on frames and cut into strips. Some of the latter were either suspended or laid horizontally across supports. None of these showed any structure at all when soaked in water and sectioned, but were homogeneous throughout. A few of the gels dried at room temperature showed slight lamellations in places on the under side of the gel near the silk, but this was not so in all cases. It is apparent from the above, that the temperature and therefore perhaps the speed of drying may be an important factor in producing this lamellated structure.

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* Hydration and growth. Carnegie Inst. Washington Pub. No. 297, 1920.

Gels of varying per cents of agar content were employed for this work, from 0.5–10 per cent. They all showed structure when dried at 70° C. A 0.25 per cent gel was tried but was too weak to set at room temperature. The following concentrations of gels, expressed in per cent content of dry powdered agar, were used: 0.5, 0.75, 1, 1.5, 2.5, 3, 4, 5, 7.5, 8, and 10 per cent. The 4 per cent gel was used to determine the point at which structure appeared as loss of water proceeded.

It seems probable that this structure must form when a certain water concentration is reached in the drying of the gel. In order to ascertain at about what concentration the formation of structure began and was complete, a 4 per cent agar gel was made and, when set, cut into pieces four by two and a half inches. Each piece was placed between a pair of tin frames which were held together by two elastic bands. Each frame had two holes in it one by one and a half inches. The whole apparatus with the agar was then put in the drier, for varying lengths of time, at a temperature of about 90° C. It was turned over at short intervals so that loss of water would be equalized on the two sides. The slices did not split during the process and were removed very easily from the frames. A portion of the pieces was cut off and put into distilled water; the other part weighed, put back into the drier and finally dried to constant weight in a calcium chloride desiccator. The part put into the water was sectioned after about twenty-four hours to see if there was any structure present.

TABLE I

PER CENT BY WEIGHT OF WATER AND AGAR IN A 4 PER CENT AGAR GEL WHEN NO STRUCTURE PRESENT

Weight of gel when removed from drier in grams	Final weight of gel in grams	Weight of water in piece when removed from drier	Per cent of water to total weight	Per cent of agar to total weight
2.254	.177	2.077	92.15	7.85
3.092	.221	2.871	92.88	7.12
2.692	.194	2.498	92.79	7.21
			92.61	7.39

TABLE II

PER CENT BY WEIGHT OF WATER AND AGAR IN A 4 PER CENT AGAR GEL WHEN STRUCTURE BEGINS

Weight of gel when removed from drier in grams	Final weight of gel in grams	Weight of water in gel when removed from drier	Per cent of water to total weight	Per cent of agar to total weight
2.331	0.181	2.150	92.24	7.76
2.351	.170	2.181	92.77	7.23
3.252	.208	3.044	93.60	6.40
1.512	.157	1.355	89.62	10.38
2.172	.172	2.000	92.08	7.92
1.959	.172	1.787	91.22	8.78
2.174	.186	1.988	91.44	8.56
			91.85	8.15

TABLE III

PER CENT BY WEIGHT OF WATER AND AGAR IN A 4 PER CENT AGAR GEL WHEN STRUCTURE IS COMPLETE

Weight of gel when removed from drier in grams	Final weight of gel in grams	Weight of water in gel when removed from drier	Per cent of water to total weight	Per cent of agar to total weight
0.306	0.235	.071	23.20	76.80
.245	.165	.080	32.65	67.35
.322	.223	.099	30.74	69.26
.289	.203	.086	29.75	70.25
.292	.225	.067	22.95	77.05
.268	.225	.043	16.04	83.96
.234	.209	.025	16.83	83.17
.215	.175	.040	18.60	81.40
.209	.196	.013	6.22	93.78
.264	.195	.069	26.14	73.86
.210	.206	.004	1.91	98.09
.279	.199	.080	28.67	71.33
.241	.219	.022	9.13	90.87
.321	.201	.120	37.38	62.62
.327	.211	.116	35.47	64.53
.306	.212	.094	30.72	69.28
.257	.177	.080	31.13	68.87
.357	.219	.038	10.64	89.36
.210	.206	.004	1.90	98.10
.235	.178	.057	24.26	75.74
			21.72	78.28

From the larger piece, which was dried to constant weight, the per cent of water in the gel at the given time was obtained. This gave a fairly accurate estimate of the relation of water content to structure but cannot be regarded as strictly quantitative, as the electric heater did not give a wholly uniform temperature over all the drier so that when the time factor is constant a slight difference in the position of the strips when in the oven might vary the concentrations in them.

The tables on page 175 show the per cent by weight of water and agar in the gels: (*a*) when there was no structure present; (*b*) when it first began to appear; and (*c*) when the structure was complete. From comparison of TABLES I and II it will be seen that the critical point for structure formation lies between about 92 per cent and 92.5 per cent water content. This, however, is not the uniform concentration throughout the piece, since the surface would of course lose water first and the water content in the center of the gel would be higher than that on the edge, as it dries from the outside in and from the edge of the frame toward the center. The structure, as one would suppose, starts from the outside of the block and goes inward. This would necessarily be the way since the surface loses water first. The structure was complete, as shown in TABLE III, when the gel contained about 21.5 per cent by weight of water.

For the purposes of this paper the agar dried to constant weight in the desiccator was assumed to contain no water. The basis of the calculations was as follows: the per cent of water by weight to the total weight of substance was calculated by subtracting the final (constant) weight, obtained by drying the pieces of agar in a calcium chloride desiccator, from the first weight, after drying varying lengths of time in the oven. The loss of weight was therefore the per cent of water in the gel when structure became apparent. The pieces gained somewhat in weight while in the desiccator due to the vapor pressure of the calcium chloride. This was not taken into account as it is a constant error in all cases.

The structure varies in the different gels but is fundamentally the same. It appears as horizontal splitting in the gel, and when very pronounced as it was in most of them, particularly those below 7.5 per cent, has somewhat the appearance of branching; the cavities being shaped in section like a biconvex lens. In the 10, 8, and 7.5 per cent gels there were fewer large cavities and more fine pores or slits (FIG. 4). In the weaker gels larger slits and fewer finer ones are seen (FIGS. 1–3).

The figures show a gradual change from the coarser to the finer structure as the per cent of agar in the gel increases. It seems as though this change probably commenced between the 5 and the 7.5 per cent gels though this has not been definitely determined. Narrower branching also appears if the gels are dried at high tempera: tures, i.e., more rapidly. Strips not dried on the frames showed structure, but curled somewhat in drying. The 2.5, 3 and 5 per cent gels after soaking in water could be peeled off in layers.

It seems possible that the structure of the gels, which when first set must be isotropic, becomes anisotropic on drying due to the strains set up within the mass. It would seem to the writer that the long axis of the particles, after drying, would be in the horizontal plane of the gel. This might cause splits in it in the horizontal and not in the vertical plane, as the water is withdrawn, as is the case here. This is, however, only a suggestion as the gels were not examined with a polariscope.

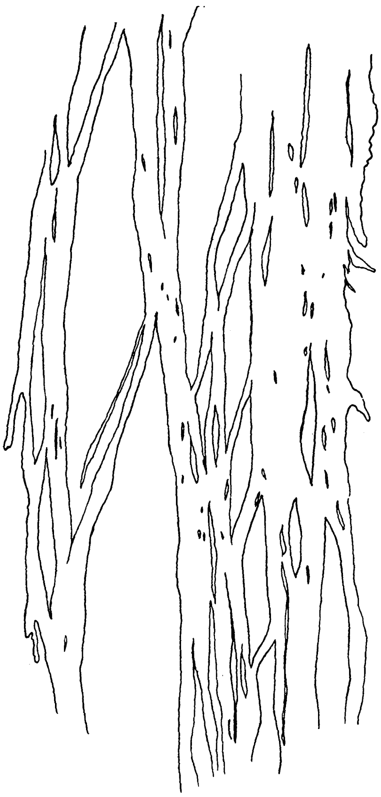
FIG. 1. A 0.5 per cent agar gel after being in water about one hour, × 57.

Ambronn* found that thin layers of agar and gelatine, when frozen and dried on glass plates, gave the appearance under the microscope of a fine network which, he says, looked almost like a section of parenchymatic plant cells. He found the walls of the meshes were like those of normal cells, having a decided double refraction, and the smallest axis of the particles lying perpendicular to the surface of the partitions caused by freezing. He did not obtain this structure after freezing and drying either egg albumin or dextrin, as the glass became covered with a uniform coat of the substance. The agar and gelatine retained their structure unless heated to a high temperature.

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* Ber. Verhandl. Königl. Sachs. Ges. Wissens. Math. Phys. Kl. 1891: 28–31.

Later Molisch,* in relation to his work on the freezing of plants, also worked on the freezing of gels. Among others he used gelatine and starch. He experimented with a 2 per cent gelatine which formed a stiff gel at room temperature. Under the microscope, at the moment of freezing, masses of ice appeared at different points which increased in size. When the ice formation had ended, a highly complicated network of gelatine remained between the ice masses. After thawing the network remained for several days as the gelatine could not adsorb the water at relatively low temperatures, and it could be fixed by treating with absolute alcohol. The starch acted in much the same manner. According to Molisch, on freezing a separation of the water and the colloid starts, in which numerous ice crystals are formed, which are enclosed by the network of the colloid. The ice is derived from the water of the gels. Such substances as white of egg, gum arabic, etc., shortly after thawing return to their original condition. Molisch compares this freezing to that of *Amoeba* and certain plant cells. He says cells may freeze like *Amoeba* and the stamen hairs of *Tradescantia*. Here the ice forms within the protoplast, which makes a network around it. Or they may freeze as *Spirogyra* and *Cladophora* do. In the latter case, the water comes out of the cell and freezes on the outside of the wall and there is a great shrinkage of the cell during the process. When cells freeze, as in the case of the colloidal gels, there is a strong dehydration since ice formation and shrinking of the protoplast go hand in hand. The spaces formed on thawing of the ice in Molisch's gelatine and starch were more spherical and less flattened than those obtained by the writer on desiccation of agar. This may be due to the shape of the ice crystals themselves, although it is perhaps unsafe to make a direct comparison, as Molisch did not work with agar.

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* Untersuchungen über das Erfrieren der Pflanzen. Jena. 1897.

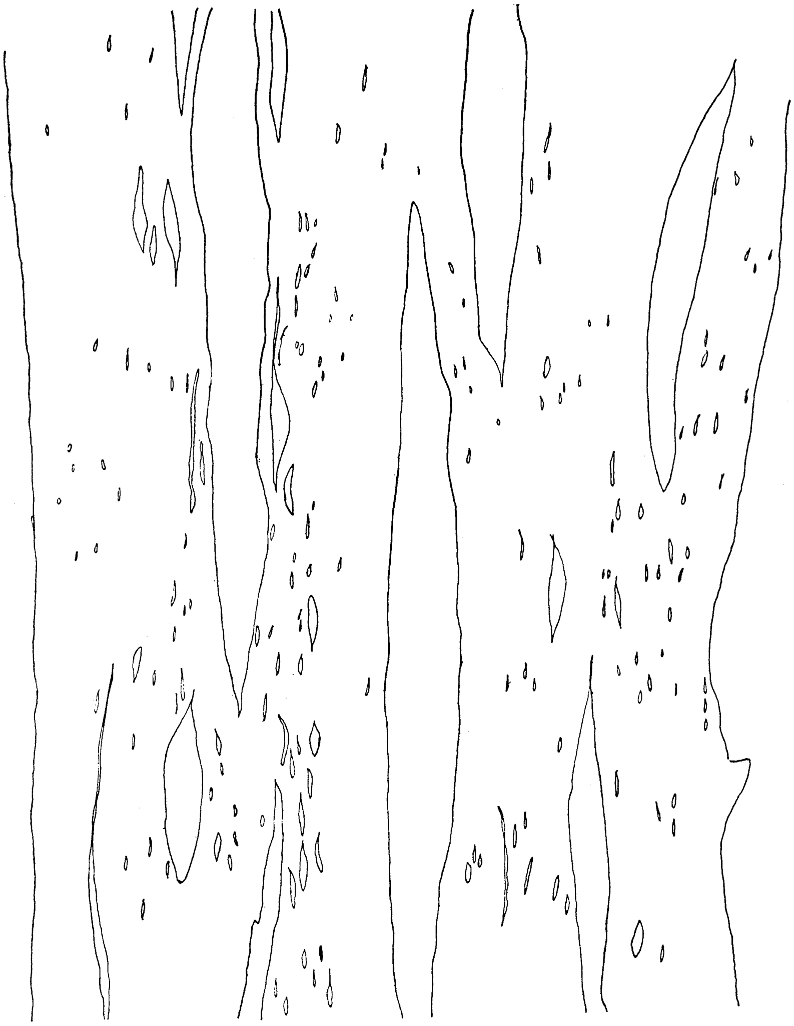

FIG. 2. A 2.5 per cent agar gel after drying at about 43° C. and soaking in water, × 57.

There seems to be a similarity between the effect of rapid dehydration due to freezing and rapid drying at higher temperatures and the structure of the gel. In both cases the water is withdrawn, but in the case of the freezing it remains as ice or water surrounded by the gel; while in the other, it is immediately removed by the high temperature at which dehydration is carried on.

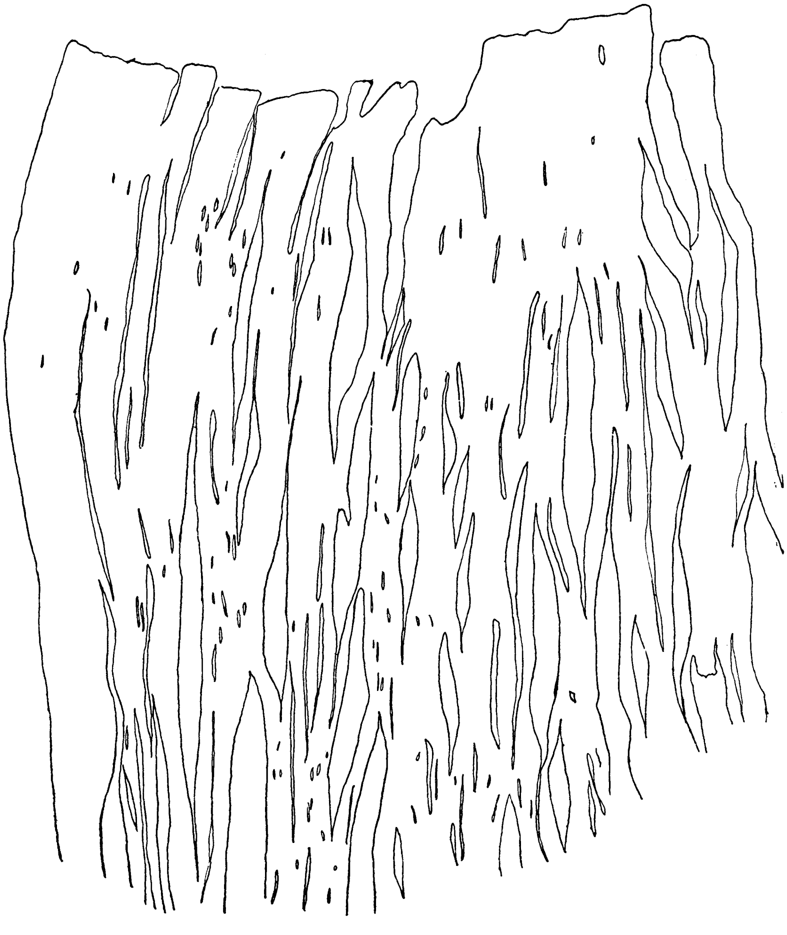

FIG. 3. A 5 per cent agar gel dried at about 70° C., after soaking in watei, × 57.

If it were possible to obtain a structural colloidal gel made of a mixture of proteins and carbohydrates, one might be able to get similar results to those obtained in plant tissues, although it might be dangerous to carry this analogy too far.

The separation of colloidal fluid from gels on standing, known as syneresis, is a phenomenon which is more pronounced the more hydrated the gel is. Fischer* accounts for the condition found in the tissues in oedema by means of syneresis, and MacDougal† considers syneresis as a means of vacuole formation after which osmotic pressure plays its part, the earlier stages being due to hydration.

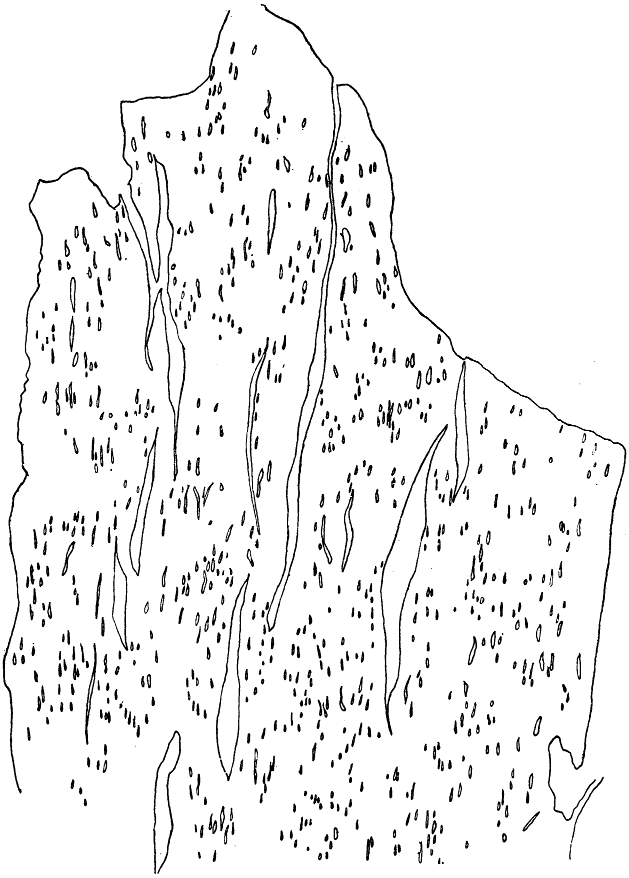

FIG. 4. A 7.5 per cent agar gel after drying at about 70° C. and soaking in water, × 57.

Zsigmondy‡ states that, if the reaction of dehydrated silicic acid gels toward hydrosols is observed under the microscope, the gel will be seen to break into small pieces. This, he says, is due to the pressure on the air in the pores of the gel together with surface tension changes. The water enters the gel quickly at first. While this goes on explosions and splittings take place in the gel and air bubbles are formed at little cracks. As the bursting of the gel and the evolution of the air proceeds, the volume of the air spaces becomes smaller and finally disappears. He does not feel that there is any evidence of explosions with water. Zsigmondy was, of course, working with a non-elastic gel which has rather different properties of hydration and dehydration than elastic gels have.

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* Oedema and nephritis. 1915.

† Growth in organisms. Science II. **49**: 599–605. 1919.

‡ Chemistry of colloids. English edition. 1917.

Zsigmondy also writes that Bütschli and later work with the ultra-microscope have shown that with silicic acid gels the dehydration does not proceed from the outside of the gel inward, but that holes are formed in the interior of the gel. These holes contain no liquid. This does not seem to be the case with agar gels, when sections of the partly dehydrated gel are viewed under the microscope, as the first slits visible are near the surface of the gel.

It appears from this work that the structure of the gels is probably due to the rapidity of the drying, which in this case varied with the temperature used. All the gels showed structure except those dried at room temperature, the majority of which showed none; a few, however, showed one or two slits. The structure is not due to the strain of stretching on silk, as pieces dried suspended or laid on trays in the oven showed the same appearance. This varied somewhat in different gels, those above 5 per cent being finer. Gels of lower concentrations when dried at temperatures of 43° C. and 70° C. did not show any great difference in structure. The per cent by weight of water and agar just before and at the beginning of structure formation is not absolutely accurate, as the gel contains a higher water concentration inside than out, owing to the fact that the drying takes place from the outside inward.

I wish to express my appreciation and thanks to Dr. Herbert M. Richards for his most helpful advice and interest.

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